CLAIMS

1. A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of:

diluting nucleic acid template molecules in a biological sample to form a set comprising a plurality of assay samples;

amplifying the template molecules within the assay samples to form a population of amplified molecules in the assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence;

comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

2. The method of claim 1 wherein the step of diluting is performed until at least one-tenth of the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required for the step of analyzing to determine the presence of the selected genetic sequence.

3. The method of claim 1 wherein the step of diluting is performed until between 0.1 and 0.9 of the assay samples yield an amplification product when subjected to a polymerase chain reaction.

4. The method of claim 1 wherein the step of diluting is performed until all of the assay samples yield an amplification product when subjected to a polymerase chain reaction and each assay sample contains less than 10 nucleic acid template molecules containing the reference genetic sequence.

5. The method of claim 1 wherein the step of diluting is performed until all of the assay samples yield an amplification product when subjected to a polymerase chain reaction and each assay sample contains less than 100 nucleic acid template molecules containing the reference genetic sequence.

6. The method of claim 1 wherein the biological sample is cell-free.

7. The method of claim 1 wherein the number of assay samples within the set is greater than 10.

8. The method of claim 1 wherein the number of assay samples within the set is greater than 50.

9. The method of claim 1 wherein the number of assay samples within the set is greater than 100.

10. The method of claim 1 wherein the number of assay samples within the set is greater than 500.

11. The method of claim 1 wherein the number of assay samples within the set is greater than 1000.

12. The method of claim 1 wherein the step of amplifying and the step of analyzing are performed on assay samples in the same receptacle.

13. The method of claim 1 wherein a molecular beacon probe is used in the step of analyzing, wherein a molecular beacon probe is an

oligonucleotide with a stem-loop structure having a photoluminescent dye at one of the 5' or 3' ends and a quenching agent at the opposite 5' or 3' end.

14. The method of claim 1 wherein the step of analyzing employs gel electrophoresis.

15. The method of claim 1 wherein the step of analyzing employs hybridization to at least one nucleic acid probe.

16. The method of claim 1 wherein the step of analyzing employs hybridization to at least two nucleic acid probe.

17. The method of claim 13 wherein two molecular beacon probes are used, each having a different photoluminescent dye.

18. The method of claim 13 wherein the molecular beacon probe detects a wild-type selected genetic sequence better than a mutant selected genetic sequence.

19. The method of claim 1 wherein the step of amplifying employs a single pair of primers.

20. The method of claim 1 wherein the step of amplifying employs a polymerase which is activated only after heating.

21. The method of claim 1 wherein the step of amplifying employs at least 40 cycles of heating and cooling.

22. The method of claim 1 wherein the step of amplifying employs at least 50 cycles of heating and cooling.

23. The method of claim 1 wherein the step of amplifying employs at least 60 cycles of heating and cooling.

24. The method of claim 1 wherein the biological sample is selected from the group consisting of stool, blood, and lymph nodes.

25. The method of claim 1 wherein the biological sample is blood or bone marrow of a leukemia or lymphoma patient who has received anti-cancer therapy.

26. The method of claim 1 wherein the selected genetic sequence is a translocated allele.

27. The method of claim 1 wherein the selected genetic sequence is a wild-type allele.

28. The method of claim 1 wherein the selected genetic sequence is within an amplicon which is amplified during neoplastic development.

29. The method of claim 1 wherein the selected genetic sequence is a rare exon sequence.

30. The method of claim 1 wherein the nucleic acid template molecules comprise cDNA of RNA transcripts and the selected genetic sequence is present on a cDNA of a first transcript and the reference genetic sequence is present on a cDNA of a second transcript.

31. The method of claim 1 wherein the selected genetic sequence comprises a first mutation and the reference genetic sequence comprises a second mutation.

32. The method of claim 1 wherein the selected genetic sequence and the reference genetic sequence are on distinct chromosomes.

33. A molecular beacon probe comprising:

an oligonucleotide with a stem-loop structure having a photoluminescent dye at one of the 5' or 3' ends and a quenching agent at the opposite 5' or 3' end, wherein the loop consists of 16 base pairs, wherein the loop has a T_m of 50-51°C and the stem consists of 4 base pairs having a sequence 5'-CACG-3'.

34. The probe of claim 33 wherein the molecular beacon probe detects a wild-type selected genetic sequence better than a mutant selected genetic sequence.

35. The probe of claim 33 wherein the molecular beacon probe detects a mutant genetic sequence better than a wild-type genetic sequence.

36. A molecular beacon probe comprising:

an oligonucleotide with a stem-loop structure having a photoluminescent dye at one of the 5' or 3' ends and a quenching agent at the opposite 5' or 3' end, wherein the loop consists of 19-20 base pairs, wherein the loop has a T_m of 54-56°C and the stem consists of 4 base pairs having a sequence 5'-CACG-3'.

37. A pair of molecular beacon probes comprising:

a first molecular beacon probe which is an oligonucleotide with a stem-loop structure having a first photoluminescent dye at one of the 5' or 3' ends and a quenching agent at the opposite 5' or 3' end, wherein the loop consists of 16 base pairs having a T_m of 50-51°C and the stem consists of 4 base pairs having a sequence 5'-CACG-3'; and

a second molecular beacon probe which is an oligonucleotide with a stem-loop structure having a second photoluminescent dye at one of the 5' or 3' ends and a quenching agent at the opposite 5' or 3' end, wherein the loop consists of 19-20 base pairs having a T_m of 54-56°C and the stem consists of 4 base pairs having a sequence 5'-CACG-3';

wherein the first and the second photoluminescent dyes are distinct.

38. A method for determining the ratio of a selected genetic sequence in a population of genetic sequences, comprising the steps of:

amplifying template molecules within a set comprising a plurality of assay samples to form a population of amplified molecules in each of the assay samples of the set;

analyzing the amplified molecules in the assay samples of the set to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence, wherein at least one-fiftieth of the assay samples in the set comprise a number (N) of molecules such that 1/N is larger than the ratio of selected genetic sequences to total genetic sequences required to determine the presence of the selected genetic sequence;

comparing the first number to the second number to ascertain a ratio which reflects the composition of the biological sample.

39. The method of claim 38 wherein the number of assay samples within the set is greater than 10.

40. The method of claim 38 wherein the number of assay samples within the set is greater than 50.

41. The method of claim 38 wherein the number of assay samples within the set is greater than 100.

42. The method of claim 38 wherein the number of assay samples within the set is greater than 500.

43. The method of claim 38 wherein the number of assay samples within the set is greater than 1000.

44. The method of claim 38 wherein the step of amplifying and the step of analyzing are performed on assay samples in the same receptacle.

45. The method of claim 38 wherein a molecular beacon probe is used in the step of analyzing, wherein a molecular beacon probe is an oligonucleotide with a stem-loop structure having a photoluminescent dye at one of the 5' or 3' ends and a quenching agent at the opposite 5' or 3' end.

46. The method of claim 38 wherein the step of analyzing employs gel electrophoresis.

47. The method of claim 38 wherein the step of analyzing employs hybridization to at least one nucleic acid probe.

48. The method of claim 38 wherein the step of analyzing employs hybridization to at least two nucleic acid probe.

49. The method of claim 45 wherein two molecular beacon probes are used, each having a different photoluminescent dye.

50. The method of claim 45 wherein the molecular beacon probe detects a wild-type selected genetic sequence better than a mutant selected genetic sequence.

51. The method of claim 38 wherein the step of amplifying employs a single pair of primers.

52. The method of claim 38 wherein the step of amplifying employs a polymerase which is activated only after heating.

53. The method of claim 38 wherein the step of amplifying employs at least 40 cycles of heating and cooling.

54. The method of claim 38 wherein the step of amplifying employs at least 50 cycles of heating and cooling.

55. The method of claim 38 wherein the step of amplifying employs at least 60 cycles of heating and cooling.

56. The method of claim 38 wherein the template molecules are obtained from a body sample selected from the group consisting of stool, blood, and lymph nodes.

57. The method of claim 38 wherein the template molecules are obtained from a body sample of a leukemia or lymphoma patient who has received anti-cancer therapy, said body sample being selected from the group consisting of blood and bone marrow.

58. The method of claim 38 wherein the selected genetic sequence is a translocated allele.

59. The method of claim 38 wherein the selected genetic sequence is a wild-type allele.

60. The method of claim 38 wherein the selected genetic sequence is within an amplicon which is amplified during neoplastic development.

61. The method of claim 38 wherein the selected genetic sequence is a rare exon sequence.

62. The method of claim 38 wherein the nucleic acid template molecules comprise cDNA of RNA transcripts and the selected genetic sequence is present on a cDNA of a first transcript and the reference genetic sequence is present on a cDNA of a second transcript.

63. The method of claim 38 wherein the selected genetic sequence comprises a first mutation and the reference genetic sequence comprises a second mutation.

64. The method of claim 38 wherein the selected genetic sequence and the reference genetic sequence are on distinct chromosomes.

65. A method for detecting a target nucleic acid, the method comprising the steps of:

separating a sample in which a target nucleic acid is present in an amount less than about 20% relative to non-target nucleic acid in said sample, thereby to form a plurality of assay samples;

exposing said assay samples to an amplification reaction designed to amplify said target nucleic acid; and

detecting said target nucleic acid as the presence of the amplification product.

66. A method for detecting a target nucleic acid, the method comprising the steps of:

providing a sample comprising X% of a target nucleic acid;

dividing the sample to produce a plurality of assay samples; wherein the ratio of target to non-target nucleic acid in at least one of the samples is greater than X%;

amplifying the single target nucleic acid to form an amplification product; and detecting the target nucleic acid as the presence of the amplification product.

67. A method for detecting a target nucleic acid in a population of non-target nucleic acid contained in a sample, the method comprising:

dividing a heterogenous sample comprising target nucleic acid and non-target nucleic acid to form a plurality of assay samples, wherein the concentration of non-target nucleic acid is at least 5 fold that of target nucleic acid;

amplifying the single target nucleic acid to form an amplification product; and

detecting the amplification product as indicative of the presence in the sample of the target nucleic acid.

68. The method of claim 66, wherein X is less than 20.

69. The method of claim 65 or 67, wherein said detecting step comprises:

exposing the amplification products to a first detectable probe which hybridizes to the target nucleic acid;

exposing the amplification products to a second detectable probe which hybridizes to the non-target nucleic acid but does not hybridize to the target nucleic acid; and

detecting the first detectable probe and the second detectable probes.

70. The method of claim 65, 66, or 67, wherein the target nucleic acid is a mutant nucleic acid.

71. The method of claim 65 or 67, wherein the non-target nucleic acid is a wild-type nucleic acid.

72. The method of claim 65, 66 or 67, wherein the sample is diluted so that between about 0.1 and about 0.9 of the assay samples yield an amplification product when amplified.

73. The method of claim 72, wherein the sample is diluted so that between about 0.1 and about 0.6 of the assay samples yield an amplification product when amplified.

74. The method of claim 65, 66 or 67, wherein one out of every two assay samples contains on average one target nucleic acid.

75. The method of claim 65, 66 or 67, wherein the target nucleic acid is a genomic DNA.

76. The method of claim 65, 66, or 67, wherein the target nucleic acid is a cDNA.

77. The method of claim 76, wherein the cDNA is generated by RT-PCR of at least one RNA.

78. The method of claim 65, wherein at least ten assay samples are analyzed.

79. The method of claim 78, wherein at least 15 assay samples are analyzed.

80. The method of claim 78, wherein at least 20 assay samples are analyzed.

81. The method of claim 78, wherein at least 25 assay samples are analyzed.

82. The method of claim 78, wherein at least 30 assay samples are analyzed.

83. The method of claim 78, wherein at least 40 assay samples are analyzed.

84. The method of claim 78, wherein at least 50 assay samples are analyzed.

85. The method of claim 78, wherein at least 75 assay samples are analyzed.

86. The method of claim 78, wherein at least 100 assay samples are analyzed.

87. The method of claim 78, wherein at least 500 assay samples are analyzed.

88. The method of claim 78, wherein at least 1000 assay samples are analyzed.

89. The method of claim 65, 66 or 67, wherein the source of the sample is selected from the group consisting of stool, blood and lymph nodes.

90. The method of claim 68, wherein the first detectable probe and the second detectable probe are molecular beacon probes that each comprise a different photoluminescent dye.

91. The method of claim 65, 66, or 67, wherein the amplifying step employs polymerase chain reaction.

92. The method of claim 90, wherein the first probe detects a wild-type nucleic acid better than a mutant nucleic acid.

93. The method of claim 90, wherein the first probe detects a mutant nucleic acid better than a wild-type nucleic acid.

94. The method of claim 65, 66, or 67, further comprising an asymmetric amplification step comprising adding a single internal primer and performing amplification.

95. The method of claim 65, 66 or 67, wherein the target nucleic acid comprises one or more mutations selected from the group consisting of base substitutions, chromosomal translocations, gene amplifications and alternatively spliced RNAs.

96. The method of claim 65, 66 or 67, wherein said method detects allelic discrimination, allelic imbalance, amplicons that are amplified during neoplastic development or rare exons.

97. The method of claim 65, 66 or 67, wherein said method detects a change in gene expression or a change in relative expression of a wild-type nucleic acid or a mutant nucleic acid.

98. The method of claim 90, wherein the first detectable probe is a first molecular beacon probe comprising a first stem and a first loop structure and having a photoluminescent dye at one of the 5' or 3' ends and a quenching agent at the opposite 5' or 3' end, wherein the first loop comprises about 16 base pairs and has a Tm of about 50-51°C, and wherein the first stem comprises about 4 base pairs having a sequence 5'-CACG-3'.

99. The method of claim 98, wherein the first loop consists of 16 base pairs and has a Tm of 50-51°C, and wherein the first stem consists of 4 base pairs having a sequence 5'-CACG-3'.

100. The method of claim 89, wherein the second detectable probe is a second molecular beacon probe comprising a second stem and a second loop structure and having a photoluminescent dye at one of the 5' or 3' ends and a quenching agent at the opposite 5' or 3' end, wherein the second loop comprises about 19-20 base pairs and has a Tm of about 54-56°C, and wherein the second stem comprises about 4 base pairs having a sequence 5'-CACG-3'.

101. The method of claim 100, wherein the second loop consists of 19-20 base pairs and has a Tm of 54-56°C, and wherein the second stem consists of 4 base pairs having a sequence 5'-CACG-3'.

102. The method of claim 65, 66, or 67; wherein said dividing step comprises diluting said sample.

103. The method of claim 65, 66, or 67, wherein the target nucleic acid comprises one or more somatic mutation.